

## WHAT IS CLAIMED IS:

- TUTTOX-05957662
1. A method for measuring ligand binding to a vanilloid receptor comprising the steps of:
    - 5 (a) forming in an aqueous solution having a pH in the range of about 7.5 to about 10.0 a liquid composition comprising a test compound, a labeled ligand, and at least a ligand-interacting portion of a vanilloid receptor protein;
    - (b) incubating the solution for a time sufficient to permit the test compound and labeled ligand to contact the vanilloid receptor;
    - 10 (c) measuring the amount of labeled ligand bound to the protein; and
    - (d) determining if the test compound bound to the receptor by observing a reduction in the amount of expected labeled ligand.
  - 15 2. The method of claim 2 wherein the ligand-interacting portion of a vanilloid receptor protein is an intact vanilloid receptor protein.
  3. The method of claim 1 wherein the vanilloid receptor is a human vanilloid receptor.
  - 20 4. The method of claim 1 wherein the pH is in the range of about 8.0 to about 9.5.
  5. The method of claim 1 wherein the pH is in the range of about pH 8.1 to about 9.1.
  - 25 6. The method of claim 1 wherein the labeled ligand is a radiolabeled ligand.
  - 30 7. The method of claim 6 wherein the radiolabeled ligand is tritiated resiniferatoxin.

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8. The method of claim 1 additionally comprising the steps after the incubating step of:
- removing unbound labeled ligand from the solution; and
- 5 isolating the receptor protein;
9. The method of claim 1 wherein the aqueous buffer further comprises a divalent cation selected from the group consisting of:
- (a) magnesium at a final concentration of between about 1 to about 10 5mM; and
- (b) calcium at a final concentration of about 0.1mM to about 2 mM
10. The method of claim 9 wherein the magnesium concentration is about 2 mM.
- 15 11. The method of claim 9 wherein the calcium concentration is about 0.8 mM
12. The method of claim 9 wherein the vanilloid receptor is a human 20 vanilloid receptor.
13. The method of claim 1 wherein the removing step comprises adding a sufficient quantity of alpha 1 acid glycoprotein to the aqueous solution to adsorb unbound labeled ligand.
- 25 14. The method of claim 1 wherein the steps are performed in order.
15. The method of claim 9 wherein the isolating step is performed before the removing step.
- 30 16. A method to measure ligand binding to a vanilloid receptor comprising the steps, in order

- TUTORIALS/55/01/2010
- (a) combining in an aqueous solution having a pH in the range of about 7.5 to about 10.0, a test compound, a labeled ligand, and vanilloid receptor protein, said protein being associated with a portion of a cell membrane;
- 5       (b) incubating the solution for sufficient time for the test compound and ligand to contact the vanilloid receptor;
- (c) adding a sufficient quantity of alpha1 acid glycoprotein to the solution to adsorb unbound labeled ligand;
  - (d) isolating the membrane from the aqueous solution;
- 10     (e) measuring the amount of labeled ligand bound to the protein in the membrane; and
- (f) determining if the test compound bound to the receptor by observing a reduction in the amount of expected labeled ligand.
- 15     17. A method to measure compound binding to a vanilloid receptor comprising the steps, in order
- (a) combining in an aqueous solution having a pH of about 8.6, a test compound, a radiolabeled resiniferatoxin, and a human vanilloid receptor-1 (VR1) protein, said protein being a portion of a cell membrane;
  - (b) incubating the solution for sufficient time for the test compound and the resiniferatoxin to contact the vanilloid receptor;
  - (c) adding a sufficient quantity of alpha1 acid glycoprotein to the solution to adsorb unbound resiniferatoxin;
- 20     (d) isolating the membrane from the aqueous solution;
- (e) measuring the amount of resiniferatoxin bound to the protein in the membrane; and
  - (f) determining if the test compound bound to the receptor by observing a reduction in the amount of expected resiniferatoxin.
- 25     18. The method of claim 17 wherein the buffer also contains a divalent cation selected from the group consisting of:
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- (a) magnesium at a final concentration of about 2mM; and
- (b) calcium at a final concentration of about 0.8mM.

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